DOI :https://doi.org/10.36378/juatika.v5i1.2731
JUATIKA
JURNAL AGRONOMI TANAMAN TROPIKA
VOL.5 NO. 1 January 2023
eissn 2656-1727
pissn 2684-785X
Hal : 82 – 95

Potential Rhizospheric Bacteria of Local Rice Rice “Cempo Laut” in Merauke

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ABSTRACT

Non-cultivated local rice is known to have high resistance to various environmental stresses. This resistance is inseparable from the role of symbiotic and non-symbiotic rhizosphere bacteria that live in the root rhizosphere. This study aims to isolate and study the potential of rhizobacteria that live around the roots of non-cultivated local rice plants. This research is an exploratory research to obtain rhizobacterial isolates that can stimulate plant growth and development, and can be applied to cultivated rice plants. Soil samples were taken from Mimi Baru Village, Jagebob District. Sampling was carried out aseptically. Isolation was carried out using the multilevel dilution method. Isolates grown in NA media were then subcultured to obtain pure isolates. The isolates obtained were then characterized morphologically according to Bergey's Manual of Determinative Bacteriology, and characterized microscopically. Based on the results of this study it can be concluded that there were 12 bacterial isolates in the local rice "Cempo Laut" which were suspected to be Bacillus and Pseudomonas.The results of this study need to be tested further to determine the effect on plant growth and development.

Keywords*: local rice, Cempo Laut, Rhizobakteri, rhizosphere, isolation*

1. INTRODUCTION

Efforts to increase production in the cultivation of food crops encourage excessive use of chemicals on agricultural land(Xiang et al., 2012;NING et al., 2017). This can increase chemical residues in the soil which can damage the soil biotic community, so that it can actually reduce plant productivity(Loks et al., 2014;Bai et al., 2020). Sustainable agriculture is an option to prevent degradation of soil quality and fertility through environmentally friendly agricultural land management by taking into account the sustainability of soil ecosystem life.(Singh et al., 2011;Finkel et al., 2017;Roberts & Mattoo, 2019). One of the efforts made inThe application of sustainable farming systems is the utilization of soil microorganisms such as bacteria, fungi and algae which are able to enhance plant growth and development(Vejan et al., 2016;Ray et al., 2020).

The rhizosphere is the area around plant roots which plays an important role in regulating plant growth and development. The root zone of this plant is one of the most complex ecosystems in the inhabited worldby bacteria, fungi, nematodes and algae(Chandran & Meena, 2021). Bacterial colonies that live in the rhizosphere are called rhizobacteria(Jha & Subramanian, 2012). Plant growth-promoting rhizobacteria (PGPR) which has been used extensively in agriculture as a biofertilizer and biological control agent to enhance plant growth, increase production,
as well as controlling diseases in plants (Hassen et al., 2016).

PGPRs that inhabit the rhizosphere of plants release phytohormones that are important for plant growth or produce bioactive compounds that can increase levels of endogenous plant phytohormones, increase the availability and absorption of nutrients through fixation and mobilization, or reduce the harmful effects of plant microorganisms.(Prasad et al., 2019). The root area provides a good environment for the growth of various biological components that interact with each other.

The rhizosphere of the rice plant has the potential for rhizobacteria to stimulate the growth and development of the rice plant and increase the activity of plant resistance to various environmental conditions.de Souza et al., (2021)succeeded in isolating 9 isolates of plant growth promoting bacteria from the rhizosphere of rice plants which have the ability to increase resistance of rice plants to cold stress. Inoculation of these bacterial isolates can increase the resistance of rice plants to cold stress in the early growth stages of plants. Isolationrhizosphere bacteria on rice plant roots carried out by(Modi et al., 2017), produced PGPR isolates that were able to produce IAA, siderophore, protease, cellulase, chitinase, HCN, dissolve phosphate, fix N2, and have antifungal activity.Sherpas et al., (2021), also obtained bacterial isolates capable of producing Indole Acetic Acid (IAA) and Phosphate solubilizing bacterial isolates from the rhizosphere of rice plants. These isolates significantly increased plant height, root length, and increased root and shoot dry weight of rice plant seedlings. Research result(Minorsky, 2019), Pseudomonas fluorescens B16 isolated from graminae roots can colonize various other plant roots and can increase plant height, number of flowers, number of fruits, and total fruit weight of tomato plants. Bacterial inoculation (Pseudomonas putida, Pseudomonas fluorescens and Azospirillum lipoferum) was able to increase the growth of rice plants, root and shoot biomass (García de Salamone et al., 2012;Sharma et al., 2014), increase the absorption of nutrients(García de Salamone et al., 2012), increase the availability of micronutrients such as Fe and Zn, and can significantly increase photosynthetic

capacity by increasing the chlorophyll content of leaves(Sharma et al., 2014).

The use of biofertilizers (biological fertilizers) is currently being widely used as an effort to maintain crop productivity and reduce the use of chemical fertilizers on agricultural land so as to maintain ecosystem sustainability.(Modi et al., 2017).According to research resultsSivojiene et al., (2021), the addition of organic fertilizers to the soil can increase the abundance and diversity of soil microorganism communities, while the type of organic fertilizer used determines the abundance of the most active types of soil microorganisms. Other research states that the use of biofertilizers can increase the diversity and abundance of microbial communities in the rhizosphere soil of maize plants.(J. Wang et al., 2021).Sustainable agriculture (sustainable agriculture) is an option to prevent a decrease in soil quality and fertility through environmentally friendly agricultural land management by taking into account the sustainability of soil ecosystem life. One of the efforts made in implementing sustainable agricultural systems is the utilization of soil microorganisms such as bacteria, fungi, and algae which are able to support plant growth and development.(García de Salamone et al., 2012;Vejan et al., 2016).

The rhizosphere around plant roots is inhabited by microorganisms that can affect plant growth and development either directly or indirectly. The root area provides a good environment for the growth of various biological components that interact with each other. The rhizosphere is a habitat for a wide variety of microorganisms. Bacterial colonies that live in the rhizosphere are called rhizobacteria(Jha & Subramanian, 2012).
The availability of nutrients in the rhizosphere is influenced by a combination of factors including soil properties, plant characteristics, and the interaction between roots and microorganisms around the roots.(Hasan et al., 2019). When plants are exposed to nutrient stress, plants will perform several mechanisms to increase nutrient availability in the rhizosphere, one of which is by changing root morphology.

Changes in root morphology can increase the affinity of nutrient transporters in the plasma membrane and exudate organic compounds such as carboxylates, phenols, carbohydrates, enzymes, and protons.(Vacheron et al., 2013;Hasan et al., 2019;Bhat et al., 2020;Grover et al., 2021). These organic compounds are used as a source of energy and carbon for microorganisms, especially groups of microorganisms that live in the rhizosphere(Chauhan et al., 2016). The chemical compounds secreted by the roots into the soil are known as root exudates(Miller et al., 2019). Chemical changes that occur around the rhizosphere can cause changes in the abundance and composition of microorganisms (Garcia et al., 2022).
Soil bacteria play a role in various biotic activities in soil ecosystems and play a role in regulating the dynamics of sustainable nutrient cycles in agriculture(Ahemad & Kibret, 2014). Soil bacteria stimulate plant growth by mobilizing nutrients in the soil, producing a number of regulators for plant growth, protecting plants from phytopathogens by inhibiting the growth of phytopathogens, improving soil structure, and playing a role in bioremediation by isolating toxic heavy metal groups and degrading compounds. xenobiotics such as pesticides(Ahemad & Kibret, 2014).
Bacteria that live in the rhizosphere and colonize plant roots, can promote plant growth through a variety of different mechanisms and are called Plant Growth Promoting Rhizobacteria (PGPR) (Sharma et al., 2014). PGPR plays an important role in increasing plant growth through various mechanisms, including increasing plant resistance to abiotic stress, facilitating the absorption of nutrients in plants through nutrient fixation, as a growth regulator in plants, producing siderhopors, producing volatile

organic compounds, producing enzymes that protect plants from attack. diseases such as chitinase, glucanase, and ACC-deaminase(Choudhary et al., 2011).

Biofertilizer is a product that is formulated to contain one or more microorganisms that can improve the nutrient status of both growth and production in plants by replacing nutrients in the soil, increasing the availability of nutrients in the soil, and or increasing access to nutrient absorption in plants.(Choudhary et al., 2011). According to(Mishra et al., 2013)Biofertiliser is a mixture of live and invisible cells that can promote nitrogen fixation, dissolve phosphates, or cellulolytic microorganisms that are applied to soil, seeds, roots or compost with the aim of increasing the number of beneficial microorganisms and accelerating the processes carried out by microorganisms thereby increasing the availability of nutrients. for easy assimilation and absorption by plants.

Plants often experience various environmental stresses such as changes in temperature (low or high), salinity, drought, acid soils, and also toxic metals, which have an impact on decreasing production.(Kang et al., 2014). Plant resistance in facing environmental stress cannot be separated from the role of rhizosphere bacteria. Under drought stress conditions, PGPR can have a positive effect in increasing germination speed, tolerance to drought stress, root and shoot weight, crop yields, and plant growth.Kang et al., 2014). Genotypes that have a high efficiency level of nutrient use can adapt to environments that have low nutrient availability(Mcdonald et al., 2013).

Some PGPR strains are capable of producing chemical compounds that can help plants deal with environmental stress by producing 1-amino-cyclopropane-1-carboxylate (ACC) deaminase. ACC deaminase is produced by Plant-growth promoting bacteria to protect plants against various abiotic stresses, including drought, salinity, heat, water stress and flooding, as well as heavy metal stress. Rhizobacteria from the genera Pseudomonas, Azospirillum, Bacillus, Burkholderia, Enterobacter, and Kluyvera have been known to have ACC deaminase activity.(Ahemad & Kibret, 2014). Pseudomonas is able to produce exopolysaccharide (EPS) which not only protects bacteria from water stress, but also plays an important role in the formation and stabilization of soil aggregates, regulates nutrient availability in plants, and water flow across roots through the formation of biofilms.(Grover et al., 2011).

Merauke Regency is a district with the largest agricultural land in the South Papua region which is directly adjacent to Papua New Guinea. The topography of Merauke Regency is lowland with a height of 0-6 m above sea level. The main agricultural commodity in Merauke Regency is rice. Based on Statistical Data for Merauke Regency in 2022 rice production in Merauke Regency in 2021 reached 349,588 tons with a harvest area of 61,670.25 Ha and a productivity of 5.67 tons/Ha (https://meraukekab.bps.go.id/publication.html). Rice cultivation in Merauke has been developed since 1939 by the Dutch(Manikmas, 2010). Currently in Merauke there is still local rice which is known to have high resistance and adaptation characteristics to various environmental conditions, one of which is Cempo Laut which has characteristics of resistance to drought stress.(Ekowati et al., 2018). Some of the research results described previously stated that the rhizosphere of rice plants is rich with a variety of microorganisms that support the growth and productivity of rice plants. The variety and diversity of microbes that make up the structure of the microbial community in the rhizosphere of the rice plant is influenced by geographical location, soil type, and also the genotype of the rice plant.(García de Salamone et al., 2012);Edwards et al., 2015;Santos-Medellín et al., 2017;Ding et al., 2019).

2. MATERIALS AND METHODS

The place

This research was carried out at the Agrotechnology Laboratory, Faculty of Agriculture, Musamus University, Merauke.The sampling location is Jagebob District which is located at 140o.040'– 140o'.080' East Longitude and 7o.060'– 8o.000' South Latitude, precisely in Kampung Mimi Baru. In this village, local Cempo Laut rice is still found which grows naturally without fertilization. Samples were taken from the rhizosphere of rice growing on lebak swamp land, samples were taken at low tide and the plants were not inundated with water.

Materials and tools

The tools used in this study included petri dishes, test tubes, Erlenmeyer, beaker glass, ose needles, autoclaves, laminar air flow (LAF), incubators, orbital shakers, vortexes, colony counters, measuring pipettes, micropipette, object glass, cover glass. . While the materials used include nutrient agar (NA), distilled water, 75% alcohol, 3% H2O2, cotton, aluminum foil, plastic wrap, resealable plastic, label paper, ice box.

Tool and Media Sterilization

All glassware that will be used is wrapped in paper and then sterilized using an oven at 170-180oC for 2-3 hours. The NA medium was thawed using distilled water as needed, then heated until dissolved. The medium was sterilized using an autoclave at 121oC with a pressure of 2 atm for 15 minutes.

Sampling

The soil used is rhizosphere soil which is in the root area of non-cultivated local rice in Mimi Baru Village, Jagebob District. Soil taken at a depth of 5-10 cm using a soil drill. Soil samples were put into sterile plastic, then stored in an ice box, then taken to the laboratory for isolation. Soil sampling was carried out aseptically.

Bacterial Isolation

Bacterial isolation was carried out by means of multilevel dilution. As much as 1 gram of soil was put into 9 ml of physiological saline (0.85% NaCl) in a test tube. The solution is homogenized using a vortex. Then a series of dilutions is made by taking 1 ml of the solution and then putting it into 9 ml of physiological saline (0.85% NaCl) and so on until a series of dilutions is obtained.10-1 – 10-7. Bacterial culture was carried out by taking 0.1 ml of the solution and then pouring it into NA medium in a Petri dish using a spread plate and incubating at room temperature for 1-2 days. The isolation process was carried out aseptically in the LAF.

Purification

Bacterial purification was carried out by taking each separate colony and then subcultured on NA medium by means of streak plates until a pure culture was obtained. The pure isolates obtained were then morphologically characterized and subjected to the catalase test, motility test, and gram test.

Morphological Characterization

Macroscopic morphological observations were carried out by directly observing the characteristics of isolated bacterial colonies using a colony counter including size, color, shape, margins, and colony elevation. Identification of morphological characteristics was carried out according to the identification book Bergey's Manual of Determinative of Bacteriology.

Catalase Test

The catalase test was carried out by mixing 3% H2O2 solution and bacterial isolates on the surface of an object glass. A 3% H2O2 solution was dripped on the surface of the object glass and then the bacterial isolates were added. Bacterial isolates were taken using a sterile toothpick and mixed with 3% H2O2

solution. Bacterial isolates that react positively will form air bubbles(Pambudi et al., 2017).

Gram test

The Gram test was carried out by dropping 3% KOH on an object glass, then adding bacterial isolates. Bacterial isolates were taken with a sterile toothpick, then mixed into 3% KOH for ± 60 seconds. Gram-negative bacteria will produce a thick suspension like mucus when the toothpick is removed (Powers, 1995 in Pambudi et al., 2017).

Motility Test

The motility test was carried out by injecting the bacterial isolate into NA media using an ose needle. Motile bacteria are characterized by the presence of bacterial growth that spreads from the ose needle puncture site after 24 hours of incubation, while non-motile bacteria will grow in the ose needle puncture area.

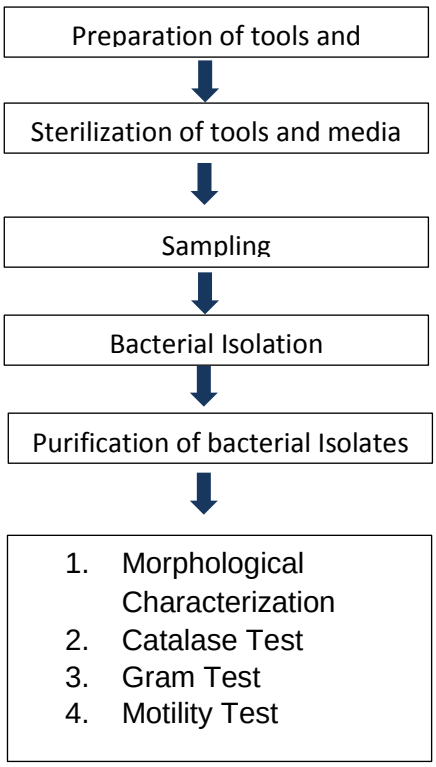

Figure 1. Research flowchart

3. RESULTS AND DISCUSSION

Morphology of Bacterial Isolate Colonies

This study succeeded in isolating 12 isolates of rhizosphere bacteria from local rice roots including isolates JSA 1, JSA 2, JSA 3, JSA 4, JSA 5, JSA 6, JSA 7, JSA 8, JSA 9, JSA 10, JSA 11, and JSA 12. The growth results of bacterial isolates on NA slanted agar medium can be seen in Figure 2. and Figure 3. as follows:

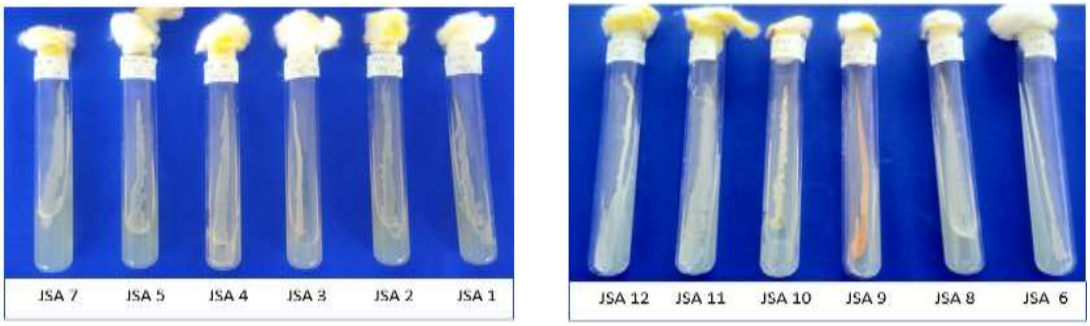

Figure 2. Isolates JSA 1, JSA 2, JSA 3, JSA 4, JSA 5, JSA 6, JSA 7, JSA 8, JSA 9, JSA 10, JSA 11, JSA isolated from the rhizosphere of local rice grown on slanted agar medium

on a petridish. The observation results of the colony morphology can be seen in Table 1 as follows:

Morphological characters were also observed based on the morphology of bacterial colonies grown on NA medium

Table 1. Morphological characteristics of bacterial isolates from the rhizosphere of local rice roots on Nutrient Agar (NA) medium

Isolate Code	Shape	edge	elevation	Size	Texture	Penam feed	Pigment tasi	Optical Properties
JSA1	*circular*	*entire*	*flat*	*moderate*	*smooth*	*shiny*	Greenish white	*translucent*
JSA2	*circular*	*entire*	*umbonate*	*small*	*smooth*	*shiny*	Greenish white	*translucent*
JSA3	*circular*	*curled*	*convex*	*punctuform*	*smooth*	*shiny*	Yellowish white	*translucent*
JSA4	*circular*	*entire*	*umbonate*	*small*	*smooth*	*shiny*	Greenish white	*translucent*
JSA5	*circular*	*entire*	*raised*	*small*	*smooth*	*shiny*	Greenish white	*opaque*
JSA6	*circular*	*curled*	*pulvinate*	*punctuform*	*smooth*	*shiny*	Yellowis white	*transparent*
JSA7	*circular*	*rhizoids*	*pulvinate*	*punctuform*	*smooth*	*shiny (slim)*	Greenish white	*translucent*
JSA8	*irregular*	*lobate*	*flat*	*punctuform*	*rough*	*shiny*	Greenish white	*translucent*
JSA9	*circular*	*curled*	*convex*	*punctuform*	*smooth*	*shiny*	orange	*translucent*
JSA10	*circular*	*curled*	*convex*	*punctuform*	*smooth*	*shiny*	Yellowish white	*translucent*
JSA11	*filamentous*	*undulate*	*flat*	*small*	*rough*	*dull*	Greenish white	*opaque*
JSA12	*filamentous*	*filamentous*	*flat*	*small*	*rough*	*dull*	Greenish white	*opaque*

Based on the observations of bacterial isolates grown with NA medium on petri dishes and on slanting agar, 4 different groups of isolates were obtained. Group 1, namely isolates JSA1, JSA2, JSA4, JSA5, JSA8, are thought to have morphological similarities to one another, then group 2 isolates, namely JSA3, JSA6, JSA 7, JSA10, are also thought to have similar colony morphology. While group 4, namely isolate JSA11, has similarities with JSA 12, and for group 3, isolate JSA9, it is a separate group. Furthermore, based on the Gram test, it showed that 10 isolates were Gram positive, including JSA1, JSA2, JSA4, JSA5, JSA6, JSA7, JSA8, JSA9, JSA11, and JSA12, while 2 isolates were Gram negative, namely isolates JSA3 and JSA 10. Motility test showed that 4 isolates were motile, namely isolates JSA3, JSA6, JSA9, and JSA10,

Based on the morphological observations of the bacterial isolates obtained, it is suspected that they belong to the genus Bacillus and Pseudomonas, namely isolates JSA1, JSA2, JSA4, JSA5, JSA8 and JSA9 which are orange in color, and isolate JSA3. JSA6, JSA7, JSA10. Various studies state that the genera Bacillus and Pseudomonas are genera of microorganisms found as PGPR in rhizosphere soils.(Manasa et al., 2017).

Based on Bergey's Manual of Systematic Bacteriology, species that are a group of the genus Bacillus have varying colony morphological

characteristics. Colony surface appearance ranges from moist and glossy (shiny) to granular and wrinkled. The shape of the colonies also shows variations, ranging from round/circular to irregular colony shapes and even spreading colonies with entire, undulate, crenate or fimbriate edges. Colony color also shows variations from white, cream, to yellowish. Some species of the genus Bacillus also produce black, brown, orange or yellow. According to researchSaha et al., (2017), several species of Bacillus isolated from rhizosphere soil are bacteria that produce yellow, orange, or brown pigments or are known as pigmentous bacteria. Most of the genus Bacillus are Gram-positive bacteria, are motile, and show positive or negative reactions in the catalase test.

According toManasa et al., (2017), 6 Pseudomonas isolates were successfully isolated from the rice rhizosphere, apart from soybeans, peanuts, sunflowers and corn. Pseudomonas isolates cultured on NA medium showed various morphological characters. The color of the colonies is pale, pale white/off white, white, dull white, to yellow, with circular/round to irregular shape with spreading growth.) and not spreading (non spreading). Pseudomonas colonies on NA medium also showed a smooth texture, with flat, convex, to raised elevations with opaque optical properties. The results of Pseudomonas isolation in the rice rhizosphere carried out byManasa et al., (2017)showed positive and negative catase test results.

The interactions that occur naturally between plant roots and the microbiome in the root rhizosphere are very important for supporting plant growth, accelerating plant growth, and suppressing disease in plants. Several studies have reported that microorganisms that colonize plant root areas, later known as Plant Growth Promoting Rhizobacteria (PGPR), can affect plant growth both directly and indirectly. The direct mechanism includes stimulating N fixation in plants, the synthesis of phytohormones and enzymes, and dissolving minerals needed by plants, while the indirect mechanism is by stimulating the inhibition of the growth of pathogens in plants (Gopalakrisnan et al., 2012).Other research states that the application of PGPR in plants can increase plant resistance to saline stress by increasing chlorophyll production and proline accumulation.(Diagne et al., 2020). According toHe et al., (2020), the application of PGPR can improve soil quality, increase enzymatic activity in the soil rhizosphere and reduce heavy metal toxicity in plants. Plant Growth Promoting Rhizobacteria (PGPR) also plays a role in increasing physiological processes and plant productivity by increasing the availability and absorption of nitrogen, phosphorus and other essential nutrients as well as the production of phytohormones.(Ahemad & Kibret, 2014;Tsukanova et al., 2017)

In addition to morphological observations, catalase, motility and Gram tests were also carried out. The test results are presented in Table 2 below:

Table 2. Catalase, motility, and Gram test results on bacterial isolates isolated from the rice rhizosphere

Isolate Code	JSA1	JSA2	JSA3	JSA4	JSA5	JSA6	JSA7	JSA8	JSA9	JSA 10	JSA 11	JSA 12
Catalase	+	+	+	+	+	+	+	+	-	+	+	+
Motility	+	+	-	+	+	-	+	+	-	-	+	+
Grams	+	+	-	+	+	+	+	+	+	-	+	+

Catalase test was performed to determine the ability of bacteria to produce catalase enzymes. Bacteria that can produce catalase enzymes will produce foam when the bacterial colonies are dripped with H2O2. The catalase enzyme is an enzyme that plays a role in catalyzing the reaction of breaking down hydrogen peroxide into oxygen and water. Catalase has been applied in various industrial fields including food processing, textile, paper, pharmaceuticals and also in the field of environmental biotechnology, namely bioremediation.(Kaushal et al., 2018). Industrialization produces toxic substances that can pollute the environment. Plants that experience environmental stresses such as salinity and extreme temperatures will increase H2O2 production(Hossain et al., 2015).Bacteria produce catalase as an antioxidant to neutralize H2O2 toxicity which increases when plants experience environmental stress. PGPR with catalase activity is able to survive in the plant rhizosphere so that it can increase plant growth(Bumunang & Babalola, 2014). According to research resultsManasa et al., (2017), P. fluorescens bacterial isolate from rice rhizosphere was able to produce catalase enzyme.Hyder et al., (2020)succeeded in isolating 8 PGPR strains from the rhizosphere of chili plants which were identified as having antifungal activity against P. capsici showing the ability to produce catalase enzymes. Catalase activity is important for bacteria to protect bacteria from hydrogen peroxide and toxic compounds produced by plants and other bacteria(Bumunang & Babalola, 2014).

Knowledge of the factors that influence the composition of soil bacterial populations is important for diagnosing environmental problems, crop productivity, and the possibility of developing bioremediation strategies.(Rai et al., 2022;Y. Wang et al., 2022). Exploration of microorganisms that live in the rhizosphere of plants is important to increase the productivity of food crops(Wu et al., 2018). Gram-positive bacteria and actinomycetes are microorganisms that are widely distributed in the soil(Princess, 2017). Gram positive bacteria control the decomposition of organic N in the soil(Enggrob et al., 2020). According to(Manasa et al., 2017), Bacillus and Pseudomonas are groups of bacteria known to inhabit the rice rhizosphere. P. fluorescens and P. putida are microorganisms that have the ability as PGPR and are effective in protecting plants from disease(Belkar & Gade, 2012).Modi et al., (2017), succeeded in isolating 12 bacterial isolates from the rhizosphere of rice plants which were identified as 6 Pseudomonas strains, 4 Azotobacter strains, and 2 Bacillus strains. These isolates have potential as PGPR, including as IAA producers, Phosphate solvents, siderophores producers, and N2 producers.

4. CONCLUSION

Based on the description in this study it was concluded that there were 12 bacterial isolates in the local rice "Cempo Laut". Further studies are needed to determine the potential of these isolates for the growth and production of rice plants.

ACKNOWLEDGEMENT

Thanks to the ladies and gentlemen of the lecturers of the Agrotechnology Study Program, Faculty of Agriculture, Musamus University and the students who were involved and helped carry out this research.

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